



## INVESTIGATION OF NOVEL PENETRATION ENHANCER LAWSONIA *INERMIS* FOR DRUG DELIVERY THROUGH NAIL PLATE

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Nail fungus infections may be very painful and can seriously harm through systemic circulation if untreated. In this study we try to find out and formulate the natural penetration enhancer (PEs). Lawsonia *Inermis* leaves were used as a penetration enhancer. To extract the penetration enhancer extraction was done with methanol and dried, which shows hundred percent penetrations across the nail plate. Human cadaver nail plate (dry weight 45.8 mg, thickness 220  $\mu$ m) defatted with chloroform: methanol (2:1) was used for penetration study. Diffusion study with the help of Franz diffusion cell with phosphate buffer saline. The transungual film F32 evaluated for the physical properties – %Drug Content  $97.1 \pm 0.03$ , Weight variation  $180 \pm 2.10$ , Thickness  $0.21 \pm 0.01$ , Flatness 99%, Folding endurance  $180 \pm 3$ , WVTR  $3.143 \pm 0.436$ , %Moisture content  $3.823 \pm 0.23$ . The drug moved across the nail plate in near to first order manner and support by the  $\log k$  value *i.e.* 0.87. The formulation with the Lawsonia *Inermis*'s extract penetrates the 2.09% more drug through nail plate. The present study can claim that the Lawsonia *Inermis* as a potent penetration enhancer for transungual delivery for which the penetration is a limiting factor.

**Key words:** Lawsonia *Inermis*, Nail Infection, Penetration Enhancer.

### INTRODUCTION

Nail fungus infections are very difficult to treat because of nail morphology and presence of infectious agent deep in nail plate. Oral drug delivery treatment is successful but limited for nail disorders with terrible side effects may be there because the need of high drug concentration needed in systemic circulation for optimal *i.e.* therapeutic concentration of drug in the site of action – nail, for wanted actions, and this therapy take a long time to treat and retrogresses are common.<sup>4,5</sup> 25 – 30% of patients relapse after treatment.<sup>1</sup> Trans ungula therapy is a magnetic choice due to its non-invasiveness, drug targeting to the site of action, eliminating systemic adverse reactions and drug interaction, increased patient compliance and per chance reduced cost of treatment<sup>6</sup>.

From the Table I one can notice that there is a significant difference between nail drug concentration and plasma drug concentration *i.e.* to maintain sufficient drug concentration in infective site (nail) one has to

increase the drug concentration in plasma also for a long time and that may cause serious side effects. One good substitute for oral drug delivery system is trans ungula drug delivery system. The absorption of therapeutic agent into the nail plate in trans ungula delivery, is highly delectable to treat the nail fungus infections. Nail permeability still quite low and restrain topical therapy. The concept of different workers that the aqueous or lipophilic vehicles do not change the drug penetration rate<sup>10,11</sup>. Penetration enhancer may help in the case of penetration problem for trans-ungula therapy. Considering history the idea of using natural material for human body has emerged numerously; and the material suitable for preparing them are termed “biomaterial”, for tranungual drug delivery very biomaterials shows good penetration within the hard keratinized nail.

### MATERIAL AND METHOD

Fluconazole was used as a model drug for study, methanol [Renkem (RFCL) limited Ranbaxy], Ethanol (Changshu Yangyuan chemical, China), Chloroform (Central drug house), Centrifuge – Teknik laboratory

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centrifuge machine, Colorimeter – labtroices model No. 12, Hot air oven (Universal). Water used in studies is of high purity demonized water (AQUOION™ TBD50). Eudragit L-100, HPMC K4M, HPMC K14M, Ethyl cellulose,  $\beta$ - cyclodextrin, cellulose acetate phthalate, 1- Butanol, phosphoric acid, 1-Propenol, 1- Dichloromethane.

### Maceration for the extract

Before going for extracting firstly, the *Lawsonia Inermis* leaves were dipped for minimum of 8 hrs in petroleum ether for removing the fatty substances and the water insoluble dirt. For the extract, we used simple maceration for the sufficient time in the suitable solvent according to the plant/pant's part. After that maceration simply filter the solution (solvent with the dissolved agents). When there was needed centrifuged the sample with 4000 – 6000 rpm for 15 minutes. Slowly separate the supernatant part with the help of pipettes and used for the further studies. The extracts after separation and centrifugation the next step was drying the extracts for further studies and for the better stability point of view as if stored the extracts in liquid form there may be the chances of any type of instability of extracts so dried form was a better option for storage.

Molecule	Plasma Drug Conc. (mg/L)	Nail Drug Conc.	Dose
Itraconazole	0.272	600 – 900 ng/g	200 mg/d 1week
Terbinafine	0.03 – 1.39	250 – 1000 ng/ g	250 mg/d
Fluconazole	0.3 – 2.7	8.5 – 9.5 $\mu$ g/g	150 mg/d

Table I: <sup>7, 8, 9</sup> - Common antifungal drugs used to treat onychomycosis.

### Collection of nail plates

For each nail plate, clinical information (age and sex) was recorded. Before using the nail plates were kept and allowed to equilibrate with the room temperature and

other conditions, cleaned with a mild liquid detergent. Thoroughly rinsed with distilled water and dried at 45°C to a constant weight. Only female fingernails (index, middle and ring finger) were use because they are already reported to be more comparable in size, weight, and thickness and more reproducible within the same donor (Lehman, personal communication). For each nail plate sample, the dry weight and thickness were measured. Thickness was measure at three points with Vernier caliper and averaged for each nail. Defatting of nail plates<sup>12</sup> Cleaned nail pieces were defatted by placing them in a beaker containing chloroform: methanol (2:1) mixture (10ml) and stirred for a period of 12 hr.

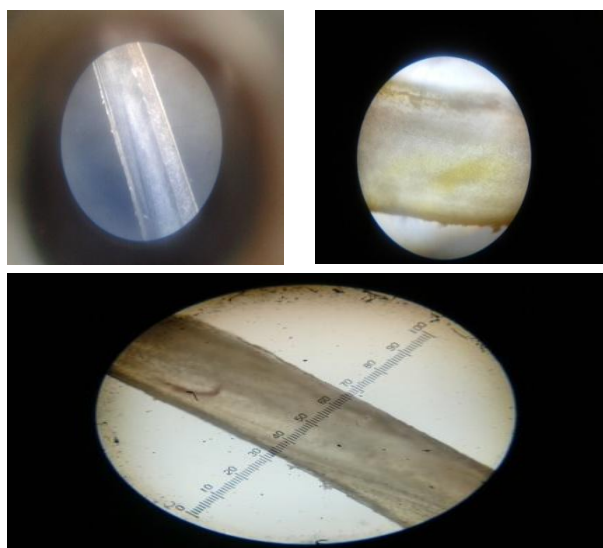


Figure I: *Lawsonia Inermis* penetration through human cadaver nail plate.

### Penetration study

For the penetration study firstly we defatted the nail plates with the solvent system chloroform: methanol (2:1) mixture. Dipped the human cadaver nail plates in the solvent system for whole night and after defatting the nail plates applied the extracts on the dorsal side of the defatted nail plates and allowed them to penetrate inside the hard compact dead keratinized nail plate, which had been considered like an impermeable structure of the human body with the normal conditions like the normal room temperature and normal

atmospheric pressure. At the 24<sup>th</sup> hour after applying the natural extract of the selected plants inspect the penetration power by observing under the compound microscope. The length covered by extract of the transverse section of the nail plate indicates the penetration power of the applied extract.

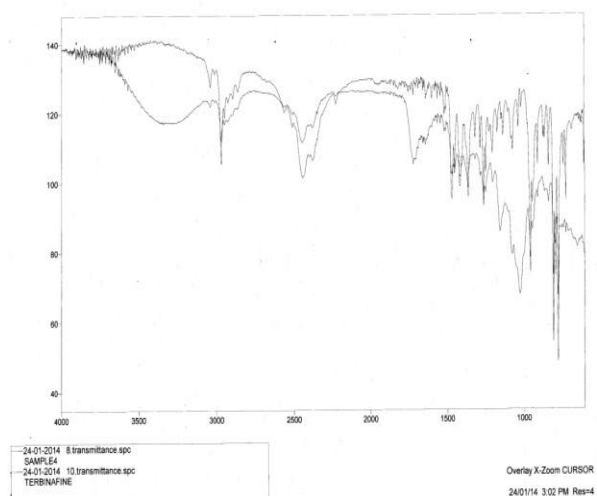
S. No.	Sample withdrawn (day)	Drug content (%) into acceptor chamber	
		Formulation without PEs	Formulation with PEs
1	1	0.00	0.00
2	2	0.00	0.00
3	3	0.00	0.08
4	4	0.00	0.35
5	5	0.12	0.67
6	6	0.23	0.96
7	7	0.37	1.43
8	8	0.62	1.98
9	9	0.93	2.50
10	10	1.2	3.29

**Table II:** Drug penetration rate determination for the formulation

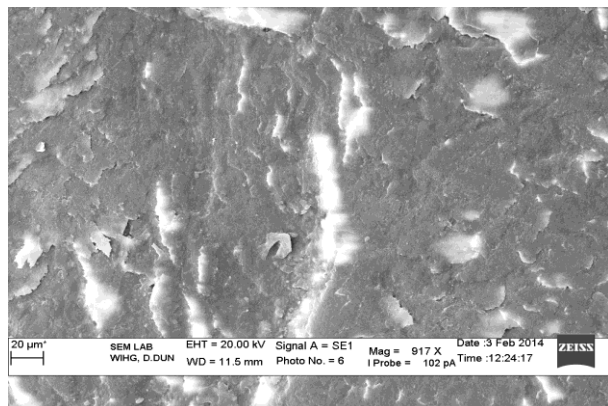
After getting the natural penetration enhancer with a great potency to penetrate the hard keratinized nail plate formulate the penetration enhancer with different stabilized pharmaceutical excipients as a transungual film. Total 56 number of formulation were formulated with Fluconazole, Eudragit L-100, HPMC K4M, HPMC K14M, Ethyl cellulose,  $\beta$ - cyclodextrin, cellulose acetate phthalate, 1- Butanol, phosphoric acid, 1- Propenol, 1-Dichloromethane. The formulated transungual film were evaluated for diffusion by the help of franz diffusion cell, with a diffusion area of 0.785 cm<sup>2</sup>. The acceptor chamber was filled with 5 ml PBS (phosphate buffer saline) at 31<sup>0</sup>C. The compatibility study was done by FTIR analysis and the possible mechanism of drug diffusion through hard keratinized nail plate concluded by scanning electronic microscopy.

## RESULT

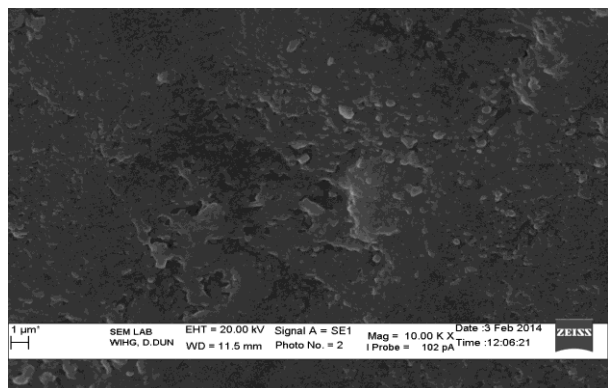
Penetration study with healthy cadaver nail plate was done at room temperature and in atmospheric pressure. The penetration study's results shows that the naturally extracted biomaterial present itself a powerful penetration enhancer in the case of hard nail plate. It penetrates almost throughout the healthy cadaver nail plate. This penetration enhancer presents himself a potent candidate as a penetration enhancer for transungual delivery therapy where penetration is a limiting factor. The transungual film F32 evaluated for the physical properties and the results were –percent Drug Content 97.1 $\pm$ 0.03, Weight variation 180 $\pm$ 2.10, Thickness 0.21 $\pm$ 0.01, Flatness 99%, Folding endurance 180 $\pm$ 3, WVTR 3.143 $\pm$ 0.436, percent Moisture content 3.823 $\pm$ 0.23. The drug release pattern shown in table no. 2, this indicated that the percent drug release of film with extracted natural penetration enhancer was greater than the film without penetration enhancer. It means that the extracted penetration enhancer was capable to migrate the drug molecules with his movement. The drug moved across the nail plate in near to first order manner and support by the Pepass “n” value *i.e.* 0.87. The overlapped FTIR spectra of the formulation and drug indicate absence of any type of incompatibility in the formulation as all major peaks were matched.



**Figure II:** Overlapped FTIR spectra for compatibility Study



Treated nail plate picture



Plan nail picture

Figure III: Scanning electroscopic microscopy picture

When we compare the SEM pictures of treated nail plate with plan nail plate we can easily conclude that there was absolutely plan surface of nail plate. The plane surface of treated nail plate told that there was simple diffusion for penetrating the drug molecules and used natural penetration enhancer in the deeper side of the nail plate. Because no sign noticed for keratolysis which may be a harmful effect for the formulation as that might be irreversible and leads to breaking of nail plate.

## CONCLUSION

The present study can claim that the *Lawsonia Inermis* as a potent penetration enhancer for transungual delivery for which the penetration is a limiting factor. *Lawsonia Inermis* extract dose not harm the nail plate and be stable for long time with normal environmental conditions. The formulation with the extract of

*Lawsonia Inermis* penetrates the 2.09% more drug in the deepest part of the human cadaver nail plate

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